(19) FEDERAL REPUBLIC OF GERMANY

GERMAN PATENT AND TRADEMARK OFFICE

(12) **PATENT APPLICATION**

(11) **DE 29 44 138 A1**

(51) Int. Cl.[3]: G 01 N 35/08

(21) Reference No.: P 29 44 138 1.52
(22) Application Date: November 02, 1979
(43) Publication Date: June 11, 1981

DE 29 44 138 A1

[stamp: illegible]

(71) Applicant

Technicon GmbH, 6368 Bad Vilbel, DE

(72) Inventor:

Assmann Prof. MD. Gerd; Helb Dr. rer. nat. Horst-Dietrich, 4400 Münster, DE; Spechl M.Sc. (Chem.) Dr. rer. nat. Werner; 3418 Üslar DE; Weber M.Sc. (Physics) Rainer 6358 Niederdorfelden, DE

An application has been filed in accordance with § 44 PatG for the examination of the patent

(54) Process and equipment for conducting analyses in automatic analysis systems by separation of deposits

DE 29 44 138 A1

FEDERAL PRINTING WORKS, BERLIN April 1981 130 024/34 8/70

X

[stamp:]
Patent attorneys
Reichel and Reichel
6 Frankfurt a. M. 1
Parkstraße 13

9365

2944138

TECHNICON GMBH, 6368 Bad Vilbel-1

Patent claims

1. Process for conducting analyses in automatic analysis systems that work according to the principle of continuous flow and by separation of deposits. The process in accordance with the invention is
characterized by the fact
that the deposit contained in a fluid to be analyzed or a deposit produced in a fluid to be analyzed is settled - if required - after an incubation period in a section of a sample stream. This section of the sample stream is led in a horizontal and linear manner and is evenly segmented with air. The process in accordance with the invention is also characterized by the fact that the sedimented deposit is removed by suction and from the remaining sample stream an aliquot portion is taken for analysis.

2. Process in accordance with claim 1 for the determination of high-density lipoproteins (HDL) particularly in body fluids by measuring the cholesterol content. The process is
characterized by the fact
that by adding a reagent comprising phosphorus wolfram acid and magnesium chloride, the very low-density lipoproteins (VLDL) and the low-density lipoproteins (LDL) are precipitated from the diluted sample. The sample is subsequently incubated, the settled deposit is extracted by suction after sedimentation and an aliquot portion is taken from the remaining fluid stream and used for the enzymatic cholesterol analysis.

130024/0034

ORIGINAL INSPECTED

FEDERAL PRINTING WORKS, BERLIN April 1981 130 024/34 8/70

X

3. Equipment for conducting the process in accordance with claim 1 with feed pipes that can be blocked by a feed pump and that can be released for the throughflow. These feed pipes are for air, washing liquids, sample and reagents. The equipment in accordance with the invention is also provided with a sampler that feeds portions of the fluid to be analyzed and washing liquids from a sample plate to the sample feed line. The equipment in accordance with the invention is provided with a confluence of the air duct into the sample line and a confluence of a reagent line into the sample line. This confluence is arranged downstream of the confluence mentioned earlier. Moreover, the equipment in accordance with the invention is provided with a mixing coil with heating bath and also a photometer and a register. The equipment in accordance with the invention is

   characterized by the fact

   that a sedimentation tube (6) is provided between the confluences of the air duct and the reagent line or after the confluence of a precipitating agent line and/or activation of a mixing coil (5) for the incubation of the precipitation. The sedimentation tube (6) comprises line sections that extend horizontally and linearly. There is also an outlet for the deposit (B) and an outlet for the supernatant fluid (A).

4. Equipment in accordance with claim 3 for implementing the process in accordance with claim 1 or 2

   characterized by the fact

   that the confluence of a precipitating agent line and a mixing coil (5) that is downstream of it and connected to it are provided between the confluence of the air duct and the sedimentation tube (6). The section from the confluence of the precipitating agent line up to the end of the mixing coil (8, 5) is made out of an antiadhesive material, particularly polytetrafluorethylene or is lined with it.

X

5. TECHNICON GMBH, 6368 Bad Vilbel-1

Process and equipment for conducting analyses
in automatic analysis systems
by separation of deposits

The invention refers to a process and equipment for conducting analyses in a continuous flow system by separation of precipitates that are either fed with the sample or arise during the course of a reaction. The analysis is then performed with a portion of the clear supernate.

The invention particularly concerns a process and equipment for determining the high-density lipoproteins (HDL) by measuring the cholesterol content wherein the low-density lipoproteins (LDL) and the very low-density lipoproteins (VLDL) are precipitated beforehand.

For 25 years hypercholesteremia is known to be the primary risk factor in the occurrence of coronary heart diseases. However, recent medical findings seem to necessitate certain modifications in the universal validity of the total serum cholesterol level as a risk factor. Diverse studies have proved the significance of HDL cholesterol. On the basis of a variety of medical findings the HDL cholesterol as opposed to total cholesterol turns out to be a protection factor against the coronary heart disease; low HDL cholesterol levels are to be considered as risk factors for coronary heart disease.

X

HDL cholesterol can be determined easily and specifically by an enzymatic reaction with subsequent color development if the other lipoproteins with low density have been separated beforehand. A range of publications suggests that a reagent comprising phosphorus wolfram acid and magnesium chloride specifically precipitates the VLDL and LDL portions in the serum, while the HDL remains in the solution.

The description of the method 'HDL cholesterol' of the company Boehringer Mannheim published in the year 1979 mentions a manual process involving the use of said reagent. This process consists of the following steps: measuring the sample, addition of a measured quantity of reagent, mixing the preparation, incubation for a definite period of time, centrifugation for a definite period of time with predetermined performance, removal of the residue, using an aliquot portion for determining the cholesterol, adding a measured quantity of cholesterol reagent, incubation for a definite period of time and photometric determination of the resulting coloring.

It is the task of the invention to replace an expensive and complex manual process of the above-mentioned type with an automatic analysis process that is characterized by the fact that unmeasured samples can be used and no pipetting and centrifugation steps are required.

The object of the invention is the process characterized in the claims 1 and 2 and also the equipment characterized in the claims 3 and 4.

The invention is elaborated in more detail on the basis of the following drawings, wherein:

Fig. 1 is a schematic illustration of the course of the stream in a sample section segmented by two blebs,

Fig. 2 is a schematic view of a sedimentation device used in the equipment in accordance with the invention and

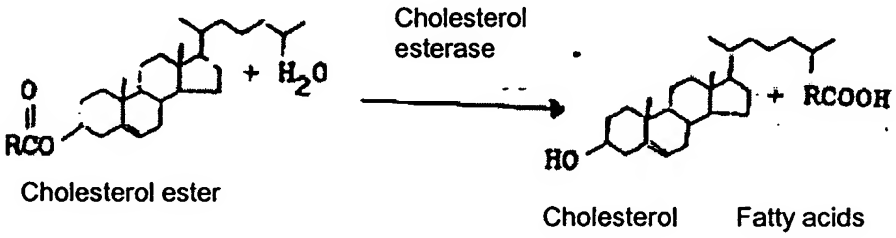
Fig. 3 is a flow pattern of the process in accordance with the invention.

The following two requirements are necessary for a sufficient sedimentation in a reasonable period of time. These requirements are fulfilled in the process in accordance with the invention:

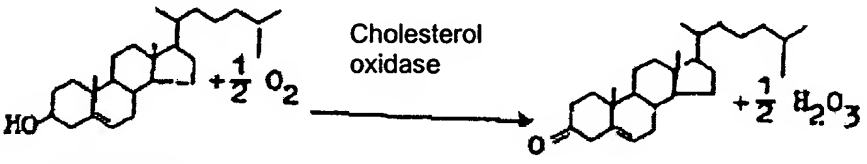
1. Even air segmentation of the stream,
2. horizontal and linear flow by avoiding any disturbance of the flow behavior, thus leading to a completely symmetrical flow distribution in accordance with figure 1.

By the bilateral restriction of the flowing fluid segment with the two blebs, the fluid layers adjacent to the wall are transported to the center of the segment and in the flow direction. Particles of higher density than that of the fluid concentrate very quickly in the lower half of the fluid segment. After the fluid is transported through the sedimentation route approximately half of the stream is extracted downward by suction and an almost clear supernate remains. Remaining residues of deposits get settled on a second route of sedimentation. From the top, an aliquot portion of the supernatant fluid is taken for the analysis and brought into contact in the known manner with an air-segmented stream of an analysis reagent and incubated. After this, the derived color reaction is analyzed photometrically.

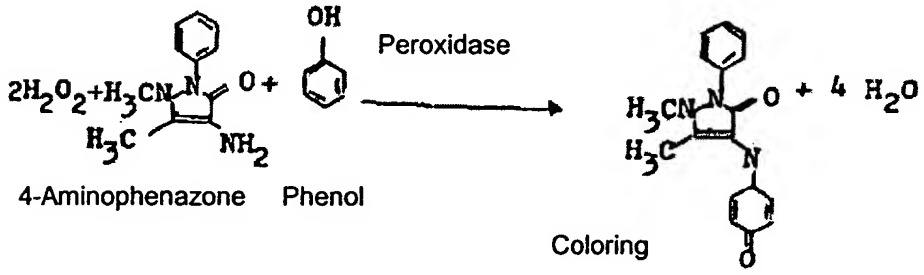
The general process in accordance with claim 1 is used in accordance with claim 2 for the automatic determination of HDL cholesterol. The complete process from taking a sample to performing the photometric analysis is fully automatic. The samples that have been drawn in are diluted, mixed with a precipitating agent and incubated, before the deposit is removed after sedimentation by suction. The deposit of the lipoproteins changes during the course of the incubation: in the beginning it is greasy and fatty and therefore sticks easily to the walls of the container if the latter is not made out of an antiadhesive material. However at the end, this deposit of lipoproteins has a solid and almost granular consistency. The cholesterol concentration is determined using the solution that is free of the deposits according to an enzymatic reaction as per the following equations:



Cholesterol ester — Cholesterol — Fatty acids



Cholesterol — Cholestenone



4-Aminophenazone — Phenol — Coloring

130024/0034

X

As per figure 3, the analysis equipment for implementing the process in accordance with the invention consists of a sampler 1, a pump 2, an analytical unit that contains a mixing coil 5, a heating bath 7 with a second mixing coil and a sedimentation tube 6, a single channel-throughflow photometer 3 with a cuvette with a length of 15 mm and a diameter of 1.5 mm and also a single channel-chart recorder 4. The feed fitting 8 and the incubation coil 5 are preferably made out of an antiadhesive material particularly polytetrafluoroethylene (PTFE) or lined with a material of this type in order to avoid an adhesion of the precipitations to the wall.

Example

The samples (serums) are placed in plastic cups that are located in the sample plate of the sampler 1. The samples are drawn in one after the other by interpositioning the washing liquid sections in such a cycle that the ratio of sample removal to the washing liquid removal amounts to 6:1 and that 60 samples are processed per hour. (This mode of operation can vary.)

The container in the sampler that contains the washing liquid is provided via the pump 2 with 2 ml water/min. Via the sample tube installed in the pump 2 and with a flow rate of 0.16 ml/min, the serum is dosed into an air-segmented stream of precipitating agent with wetting agent (1.0 ml/min) via a feed point 8 made of polytetrafluoroethylene (PTFE). The air tube delivers 0.42 ml air/min. The precipitating agent is composed of the following:

0.5% phosphorus wolfram acid
6.25 mM magnesium chloride
20 mM sodium hydroxide
pH 7.1
5 ml Polyoxyethylene lauryl ether (Brij 35) per 1000 ml

After the incubation in the PTFE mixing coil 5 with 25 windings, the stream flows through the sedimentation tube 6 with 1 winding (fig. 2). The concentrated deposit B is extracted downward by suction at the rate of 0.8 ml/min. Finally, from the supernate, a portion A of the fluid and the blebs is removed in a quantity of 0.16 ml/min and dosed to an air-segmented stream of cholesterol reagent that is incubated in a heating coil at 37°C. The optical density of the coloring resulting in the enzymatic reaction with the sample is measured in the throughflow photometer 3. The result appears on the chart reader 4.

Wa/Gu

X

- Blank Page-

Number: 29 44 138
Int. Cl.[3]: G 01 N 35/08
Application Date: November 2, 1979
11 Publication Date: June 11, 1981
2944138

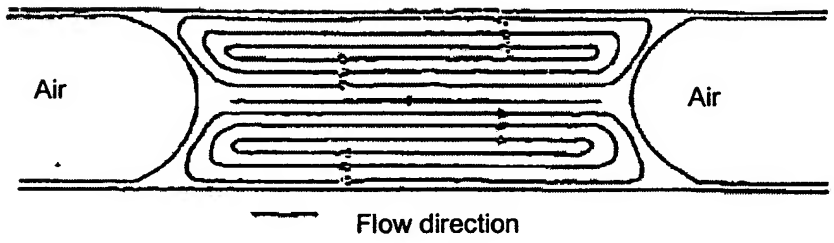


FIG. 1

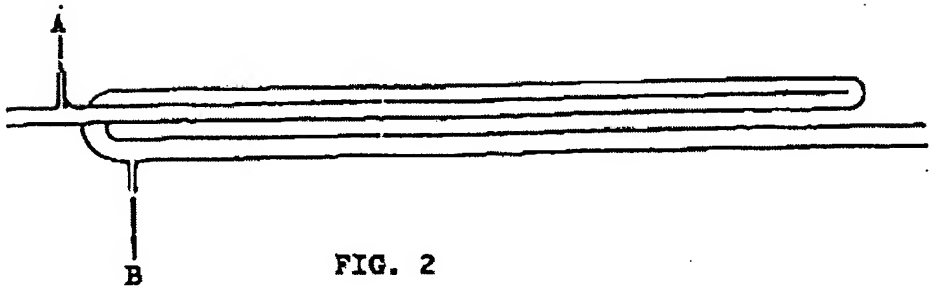


FIG. 2

X

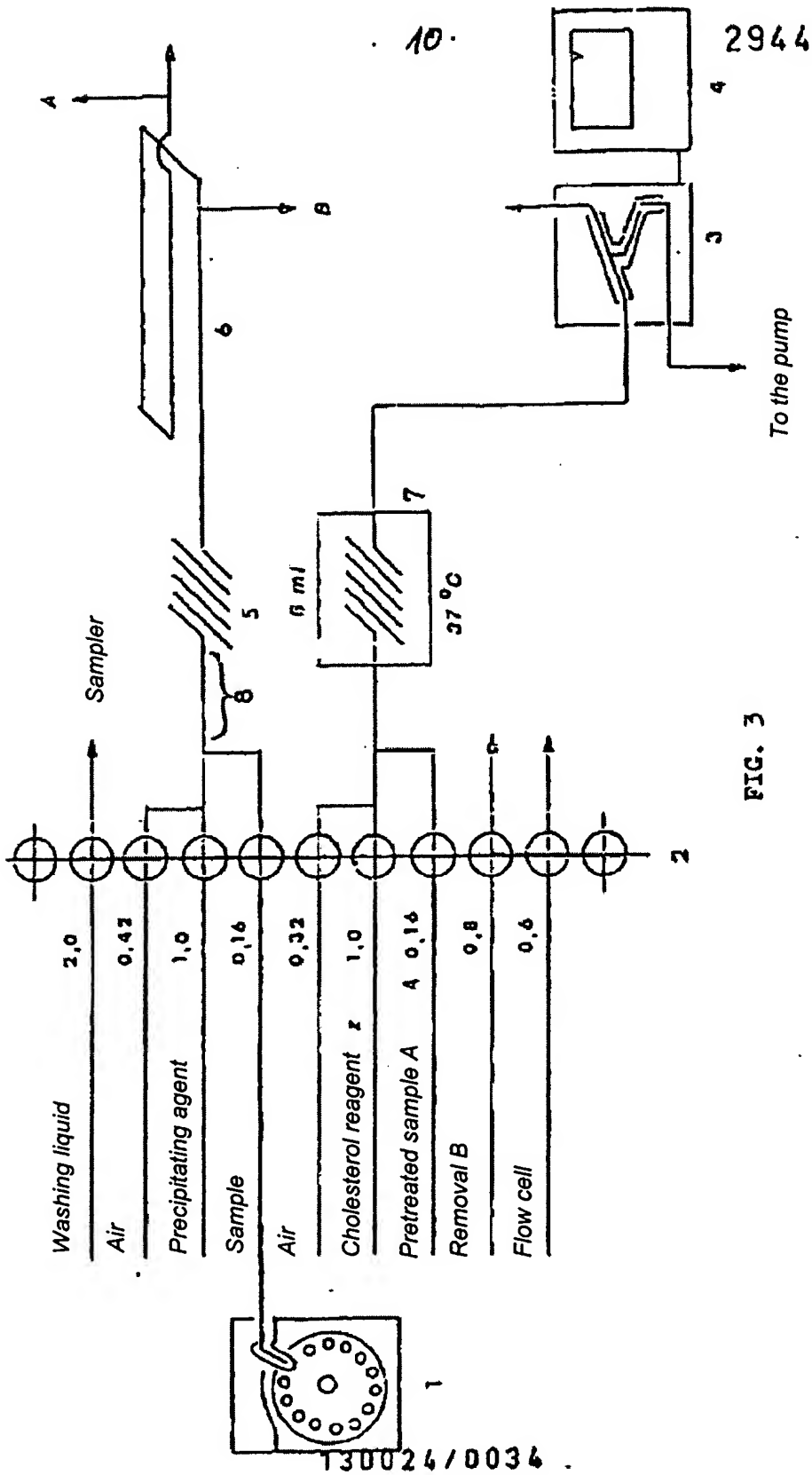

A
B
4
3
6
To the pump
7
6 ml
37 °C
5
8
Sampler
2
1
Washing liquid
2,0
Air
0,42
Precipitating agent
1,0
Sample
0,16
Air
0,32
Cholesterol reagent
1,0
Pretreated sample A
0,16
Removal B
0,8
Flow cell
0,6

FIG. 3